Organic Mass Spectrometry, 1970, Vol. 3, pp. 1187 to 1213. Heyden & Son Limited. Printed in Northern Ireland

MASS SPECTROMETRY IN STRUCTURAL AND STEREOCHEMICAL PROBLEMS—CXCIV:*

THE MASS SPECTROMETRIC FRAGMENTATIONS OF STEROIDAL SAPOGENINS†

WILLIAM H. FAUL‡ and CARL DJERASSI

Department of Chemistry, Stanford University, Stanford, California 94305, USA

(*Received* 16 *February* 1970; *accepted* 23 *March* 1970)

Abstract—The characteristic mass spectral fragmentation patterns of the basic structure of the steroidal sapogenin, (25R)-5α-spirostan, have been elucidated through the preparation of analogs with deuterium labels at positions 11, 12, 14, 15, 16, 17, 20, 21, 23, 24, 25, 26 and 27. In addition, the effects of a change of stereochemistry at positions 14 and 20, of the introduction of oxygen-containing functionalities mostly in ring F, and of the incorporation of olefinic unsaturation have been determined through synthesis of many examples.

THE CHARACTERISTIC electron-impact induced fragmentation of steroid hydrocarbons, a subject of considerable study in this laboratory,[2] is greatly affected by introduction of various functionalities.[3] The steroidal sapogenins, a naturally-occurring and industrially important class, are unique in that two oxygen atoms, incorporated as a spiroketal, are part of the basic, or 'naked', structure (25R)-5α-spirostan (I). The discovery of the enormously effective charge-localizing effect of the ethylene ketal grouping[4] as well as the general interest in sapogenins and their structure elucidation prompted an early study from this laboratory,[5] the results of which have been used in the proof of structure of nautigenin (II),[6] igagenin (III),[7] jurubidin (IV),[8] paniculidin (V),[8] neochlorogenin (VI),[9] paniculogenin (VII),[9] and 23R-bromo-(VIII) and 23S-bromo-(25R)-5α-spirostan (IX).[10]

The original rationalizations[5] of the mass spectrometric fragmentation patterns were formulated through the technique of 'functional group comparisons' and were done without recourse to deuterium labeling and at 70 eV only. The present publication describes the results of deuterium labeling [11] at positions 11, 12, 14, 15, 16, 17, 20, 21, 23, 24, 25, 26 and 27, which, coupled with low voltage and high resolution measurements, have shed considerable light on the mode of fragmentation of this system. In addition, the effect of changes in stereochemistry, of the introduction of certain oxygen functionalities and of the incorporation of olefinic unsaturation has been determined.§ The synthesis of the majority of the compounds has been described elsewhere.[11]

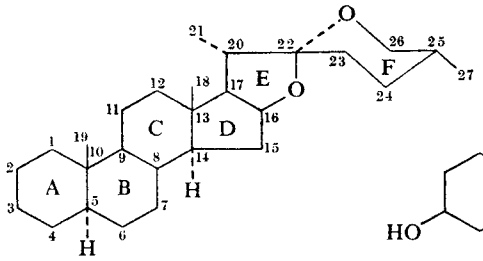
In terms of complexity or number of peaks, the mass spectrum (Fig. 1) of (25R)-5α-spirostan [3-deoxytigogenin (I)] lies midway between that of the relatively complicated androstane[2a] and cholestane[2b] and the quite simple 3-ethylene ketal derivative

* For part CXCIII, see ref. 1.

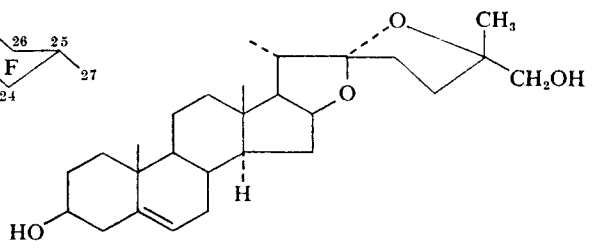
† Financial assistance from the National Institutes of Health (Grant No. AM-12758 and GM-06840) is gratefully acknowledged.

‡ Taken from the PhD thesis of W. H. F., NIH predoctoral research fellow, 1966 to 1970.

§ For a summary of some of our findings, see C. Djerassi, *Pure Appl. Chem.* in press.



(I)



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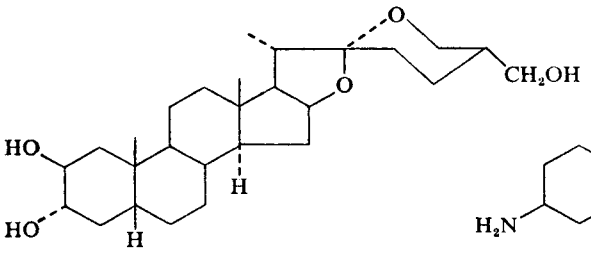

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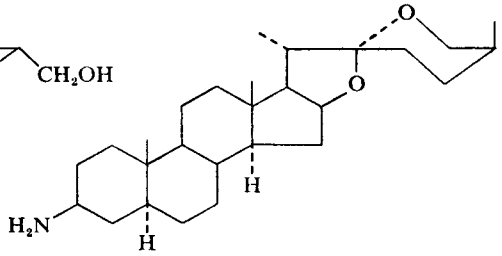

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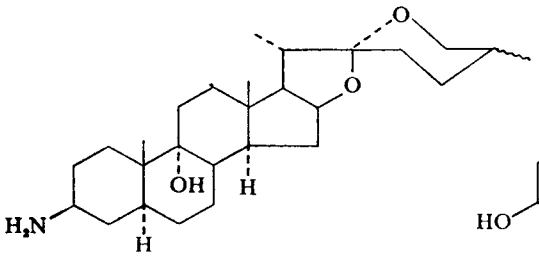

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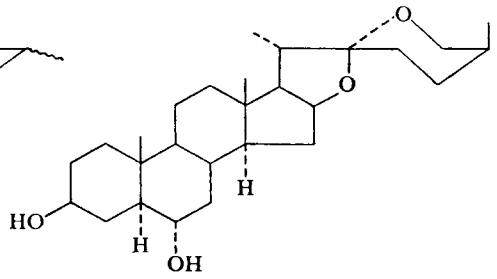
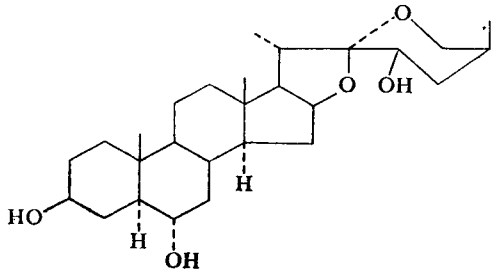
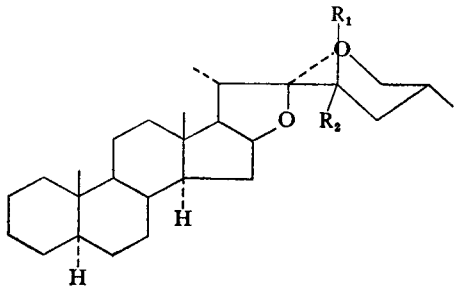


(VI)



(VII)



(VIII) $R_1 = Br$; $R_2 = H$
(IX) $R_1 = H$; $R_2 = Br$

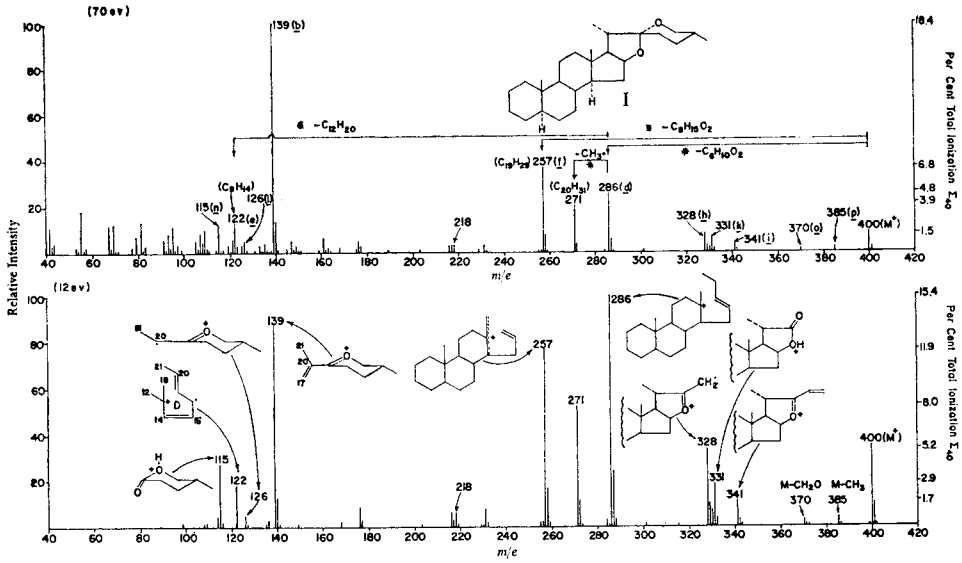
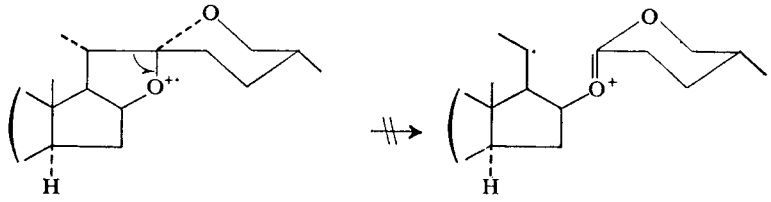


FIG. 1. Mass spectra (70 and 12 eV) of (25R)-5α-spirostan (I).

of the same species. Attention has been called[12] to the fact that the spiroketal rings E and F occupy perpendicular planes, a situation which seems to make certain bond fissions of molecular ions with the charge on one of the oxygen atoms more favorable than others due to orbital overlap. For example, no fragmentation path can be rationalized as starting with fission of the 20-22 bond (I → *x*). Therefore, one would



(I) (*x*)

expect that the overall decomposition modes of the sapogenin spiroketal system would be different from those of the ethylene ketal. In addition, this situation may also be one of the reasons that charge localization about the spiroketal oxygens in I is not as great as in the ethylene ketal[4] leading to the increased importance of pure hydrocarbon peaks (*m/e* 122, 257, 271, 286 in Fig. 1) in the low voltage spectrum. Such is not the case in the simple ketal. In fact, if one makes a direct comparison of the 'hydrocarbon' *vs.* spirostan *vs.* ketal systems by examining the spectrum of (25R)-5α-spirostan-3-ethylene ketal (X, Fig. 2), one sees a somewhat unexpected gain in importance of the hydrocarbon-cleavage peaks 315, 329, 344 (corresponding to 257, 271, 286 in Fig. 1) in the low voltage spectrum. A possible rationalization may be that initial fission of the 13-17 bond may release even more strain than in androstane or

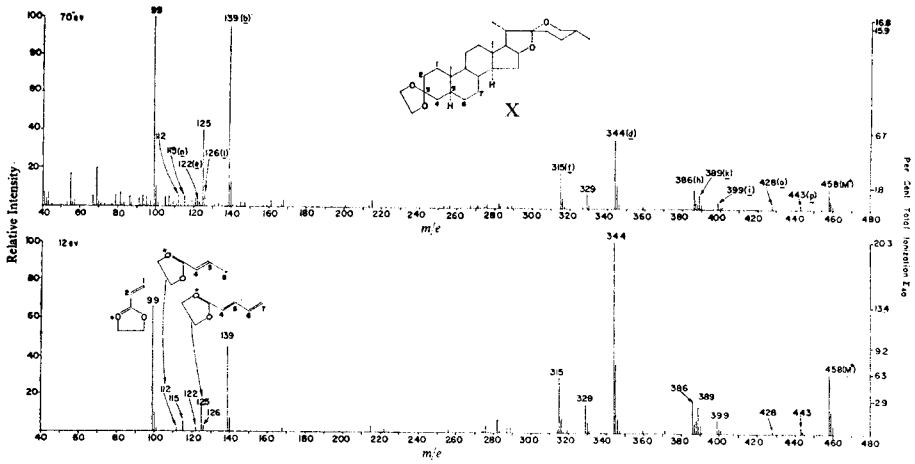
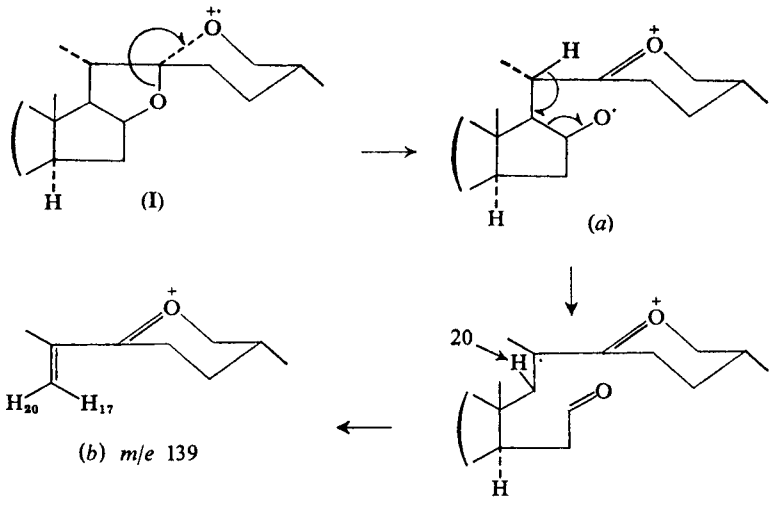


FIG. 2. Mass spectra (70 and 12 eV) of (25R)-5α-spirostan-3-ethylene ketal (X).

cholestane, principally due to the additional five membered E ring, and hence favors molecular ions of type *c* which would lead to hydrocarbon fragments.

The results of the extensive deuterium labeling[11] of (25R)-5α-spirostan (I) are summarized in Table 1 and all are consistent with the originally proposed[5] mechanism (I → *a* → *b*) for the formation of *m/e* 139, the most characteristic peak of the steroidal sapogenin and one which remains important at 12 electron volts (Fig. 1). The 1,2-shift



of the C-20 hydrogen to C-17 is, of course, not amenable to deuterium labeling and is invoked only to rationalize what otherwise would be the cleavage of two bonds connected to the same carbon atom.

The peaks at *m/e* 122, 257, 271 and 286 are of special interest since they are of a purely hydrocarbon nature and gain in importance in the low voltage spectrum. High resolution measurements indicate that 257 and 271 are $C_{19}H_{29}$ and $C_{20}H_{31}$, respectively,

TABLE 1. SHIFTS[a] OF MASS SPECTRAL PEAKS IN DEUTERATED ANALOGS OF (25R)-5α-SPIROSTAN (I)

(25R)-5α-spirostan	% isotopic purity[a]	400 $[M]^+$	385	370	341	331	328	286	271	257	139	126[d]	122[d]	115[d]
11,11-d_2	1 d_0 8 d_1 91 d_2	402	387	372	343	333	330	288	273	259	139	126	122	115
12,12-d_2	4 d_0 44 d_1 49 d_2 3 d_3	402	387	372	343	333[e]	330[e]	288[a]	273[a]	259[a]	139	126	124	115
14β-d_1[b]	14 d_0 79 d_1 7 d_2	401	386	371	342	332	329	287[a]	272[a]	257 (23%) 258 (77%)[a]	139	126	123[e]	115
15,15-d_2	34 d_1 59 d_2 7 d_3	402	387	372	343	333	330	288[a]	273[a]	259[a]	139	126	123	115 (93%) 116 (7%)[a]
16-d_1	73 d_1 26 d_2 1 d_3	401	386	371	342	332	329	287	272	258	139	—[e]	123	115
15,15,17-d_3[h]	10 d_0 30 d_1 31 d_2 21 d_3 6 d_4 2 d_5	403	388	373	344	—[e]	—[e]	289[a]	274[a]	259[a]	140[a]	126[a]	124[a]	115 (40%) 116 (60%)

TABLE 1 (*continued*)

(25)-5α-spirostan	% isotopic purity[a]	400 [M⁺]	385	370	341	331	328	286	271	257	139	126[d]	122[d]	115[d]
20-d_1	24 d_0 75 d_1 1 d_2	401	386	371	342	332	329	287	272	257	140	127	123	115
21-d_1	2 d_0 98 d_1	401	386	371	342	332	329	287[a]	271 (40%) 272 (60%)[a]	257[a]	140	127	123	115
23ξ-d_1	7 d_0 93 d_1	401	386	371	341 (67%) 342 (33%)[a]	331	329	286	271	257	140	127	122	116
23,23-d_2	4 d_1 90 d_2 6 d_3	402	387	372	341 (39%) 342 (61%)[a]	331	330	286[a]	271	257	141[a]	128	122[a]	117[a]
24ξ-d_1	98 d_1 2 d_2	401	386	371	342	331	328	286	271	257	140	127	122	116

24*S*,25-d_2	97 d_2 3 d_3	402	387	372	342	331 (89%) 332 (11%)[a]	328[a]	286	271	257	141	128	122	117
26ξ-d_1	2 d_0 97 d_1 1 d_2	401	386	370	341 (76%) 342 (24%)[a]	331[a]	328[a]	286	271	257	140[a]	127	122	116
27-d_1[c]	1 d_0[c] 90 d_1 8 d_2 1 d_3	401[c]	386[c]	371[c]	341[c]	331[c] (25%) 332 (75%)[a,i]	328[a]	286[c]	271[c]	257[c]	140	127	122	116[a]

[a] Isotope purity values are corrected for naturally occurring ^2H, ^{13}C and ^{18}O and are accurate to ±3%. Where noted, shift value was calculated in the same manner.

[b] The method of preparation[15] gives only the 14β-compound. Comparison of the mass spectra of the unlabeled 14α- and 14β-compounds I (Fig. 1) and XII (Fig. 3) shows that they differ only in a quantitative manner.

[c] The 27-label is actually[15] the 5-en-3β-ol (diosgenin), $[M]^+ = 414$. Certain of the values have been reduced by 14 mass units to conform to the rest of the table.

[d] Isotopic purity is easier to discern from 12 eV spectrum.

[e] Poor isotopic purity of the sample makes the shift assignment uncertain.

[f] The extra deuterium is at C-23 as determined by its mode of preparation. See ref. 11.

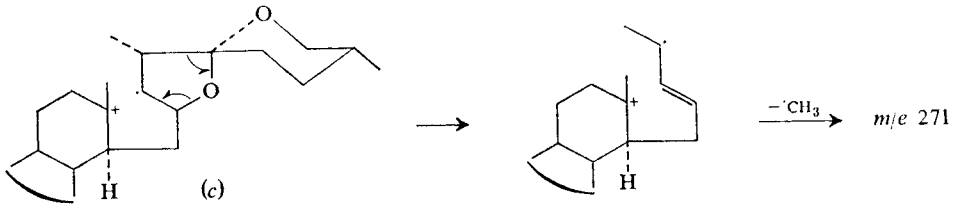
[g] Peak of small intensity split between *m/e* 126 and 127. Peak heights do not correspond to that of the unlabeled material.

[h] A detailed discussion of the results gleaned from this label appears in the text.

[i] The actual shift is *ca.* 25%. The given value reflects the fact that there is at least a 2:1 ratio in favor of a hydrogen (i.e. unlabeled) shift since the label is CDH_2-27 rather than CD_3-27.

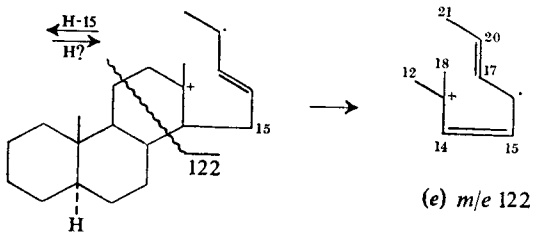
and, since there is a metastable peak for the transition *m/e* 286 → 271, the composition of *m/e* 286 is $C_{21}H_{34}$. There are also unambiguous metastable ions for the formation of *m/e* 286 and 257 from the molecular ion *m/e* 400 and one for *m/e* 286 → 122 (C_9H_{14}).

The formation of these four species can be rationalized as starting from the molecular ion *c*, a type of ion radical which also plays a very important role in the mass spectrum of cholestane.[2b] Though formation of *m/e* 286 (*d*) is easily rationalized



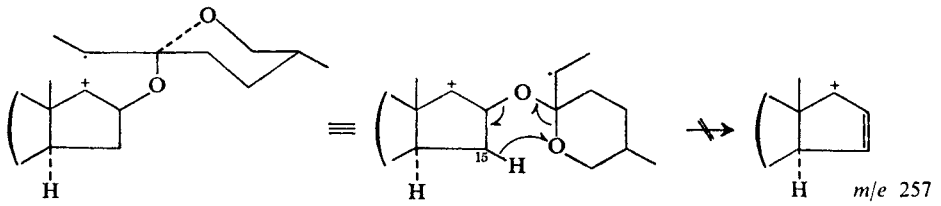
(*d*) *m/e* 286

and is consistent with the labeling data, no structure can be drawn for the next lower homolog, *m/e* 271, since the labels indicate that the C-21 methyl group is lost 40% of the time and that the rest of the loss comes from expulsion of CH_3-18 and/or CH_3-19. Furthermore, in the formation of *m/e* 122, one hydrogen is lost from C-15 and one is gained from somewhere else (an unlabeled position) in the molecule. Without recourse to additional labeling data, it seems prudent only to indicate *e* as a possible structure for *m/e* 122.



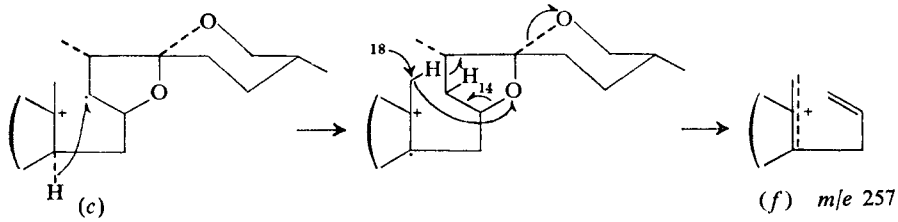
(*e*) *m/e* 122

Since the C-15 label is retained in the fragment of mass 257, the original mechanism[5] is definitely eliminated from consideration since one C-15 deuterium would be lost in that process. Another conceivable candidate for the hydrogen transfer is C-18 which

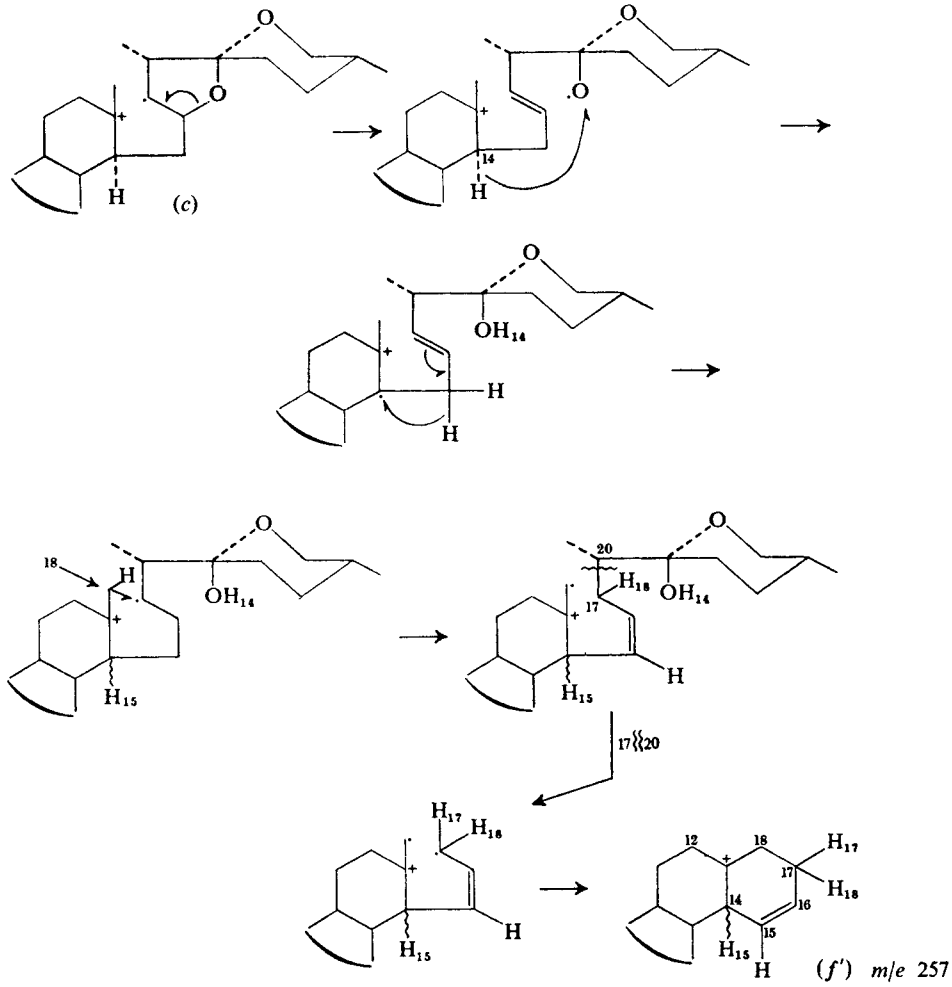


has not been labeled due to synthetic difficulties.[11] Though it must stand as speculation, the following path (*c* → *f*) has the virtue of being triggered by an internal hydrogen transfer from C-14 in the molecular ion *c*—a sequence actually documented[2b] through

deuterium labeling in the genesis of the important *m/e* 217 peak in the cholestane series. Such a process requires retention of the C-14 label and is found to be so to an extent of 77%. The 23% loss of the C-14 label also can be rationalized as starting

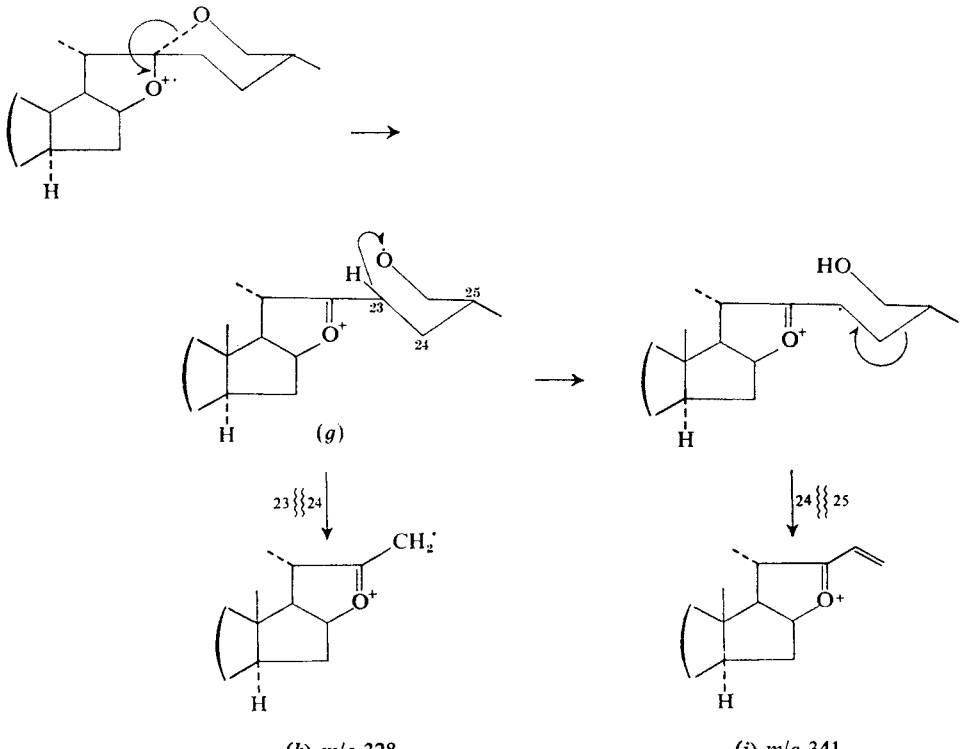


from *c* (sequence $c \rightarrow f'$) in which the C-18 hydrogen has been retained and the C-14 hydrogen lost.



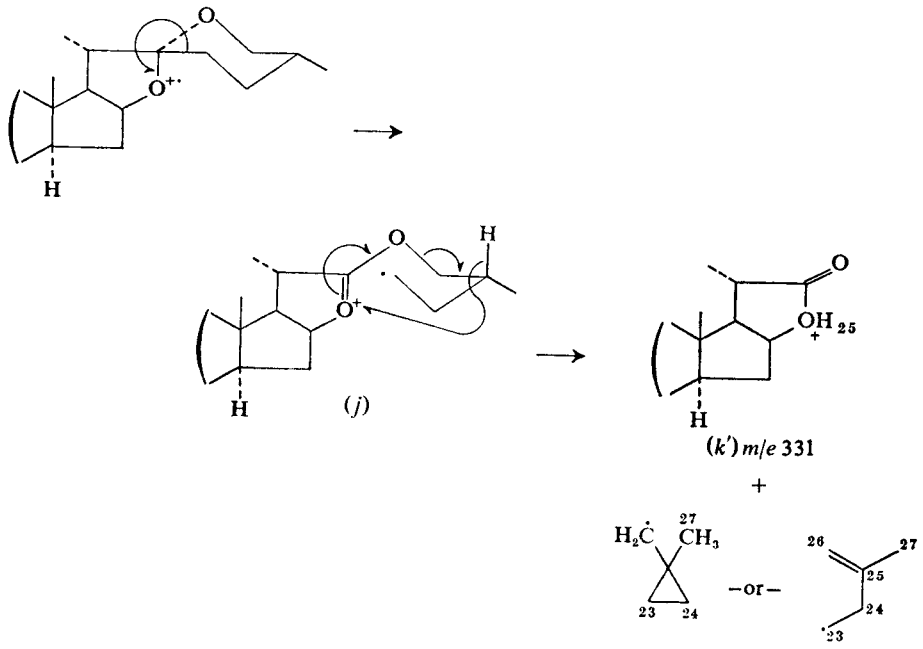
Of the triplet of small peaks *m/e* 216, 217 and 218, the latter two, though not diagnostic in the sapogenin spectrum (Fig. 1), are the counterparts to the important and interesting *m/e* 217 and 218 peaks in the spectra of cholestane and androstane[2] and may be assumed to be formed in the same manner. Their low intensity, even at 12 eV, is noteworthy.

The peaks associated with the opening of ring F are the ones at *m/e* 341, 331 and 328. Fission of the ring F oxygen, C-22 bond with charge retention in ring E yields *g*, which leads either to *h* (*m/e* 328) by direct cleavage of the 23, 24 bond or to *i* (*m/e* 341) through transfer of the C-23 hydrogen to the alkoxy radical with concomitant 24, 25 bond scission. In the latter case, the pathway explains the major fragmentation scheme indicated by the deuterium labels. The retention of 33% of the label in the 23ξ-deuterio analog can be explained by stereospecific hydrogen transfer in a 23R and 23S-deuterio mixture, the label having been introduced in a nonspecific manner,[11] but a 39% loss of both labels in the 23,23-dideuterio analog remains a mystery as does the 24% retention of the label at C-26 (see Table 1).

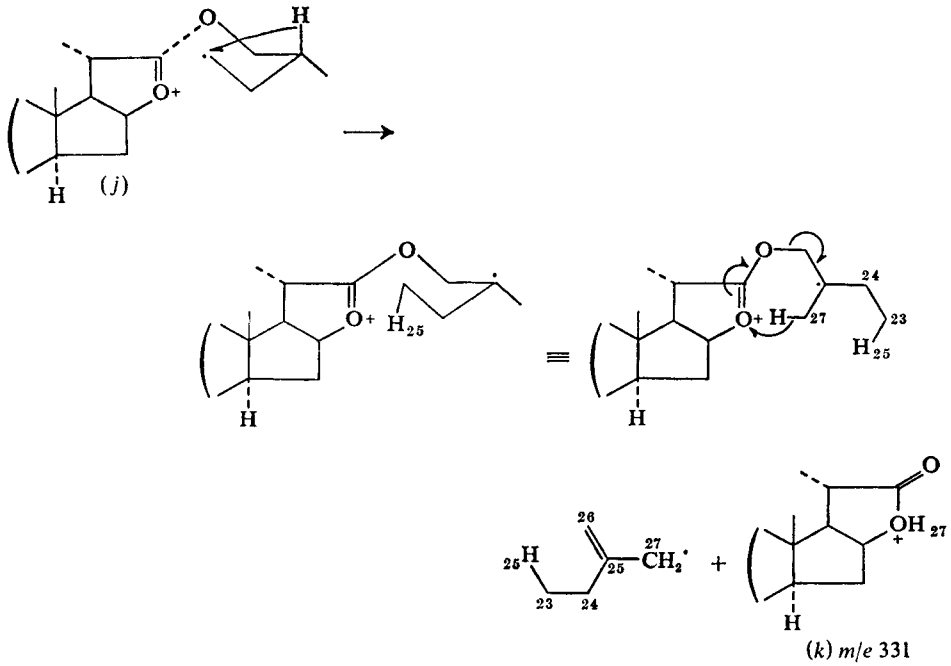


(*h*) *m/e* 328 (*i*) *m/e* 341

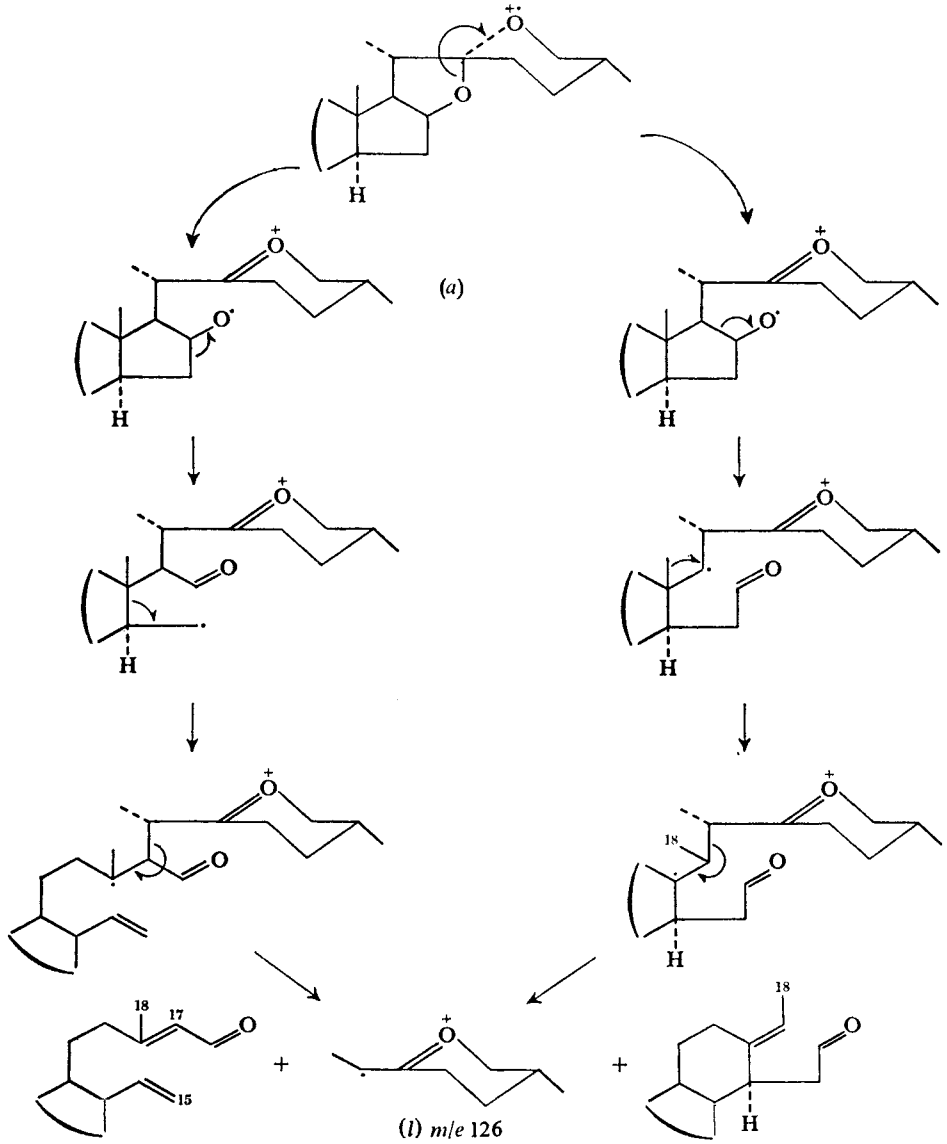
The original[5] mechanism for the formation of *k'*, *m/e* 331, included transfer of the C-25 hydrogen through a six-membered transition state in the molecular ion *j*. The neutral product would then be the cyclopropane-containing radical or a homoallylic primary radical. The labeling shows, however, that this pathway accounts for only a part (<25%) of the total, the majority being due to transfer of the C-27 hydrogen



leading to the species k rather than k'. Apparently, the driving force for this alternative path is the production of an allylic radical rather than the cyclopropane or homoallylic radical as the neutral species.



The peaks associated with opening of ring E are *l* (*m/e* 126) and *n* (*m/e* 115); both are more evident at low voltage than at the usual 70 eV. The former peak, while relatively unimportant in (25R)-5α-spirostan (I, Fig. 1), becomes more intense in certain derivatives.* Either of two pathways can lead from the molecular ion *a* to *l* (*m/e* 126). The chief difference is in the neutral species and hence is not amenable to differentiation by deuterium labeling.



* See the spectra of the 12-(XX, Fig. 12) and 15-(XXI, ref. 5) keto derivatives which are discussed later in the text.

Since the isotopic purity of the 15,15,17-d_3 analog is so poor (see Table 1), the final confirmation of the mode of formation of *n* (*m/e* 115) had to wait until the rationalizations for the formation of the other main peaks were secure. We propose that *n* (*m/e* 115) is formed from molecular ion *m* through a six-membered transition state involving the C-17 hydrogen.* Table 2 gives the isotopic composition associated with the main fragment peaks in the mass spectrum of 15,15,17-d_3-(25R)-5α-spirostan.[11]

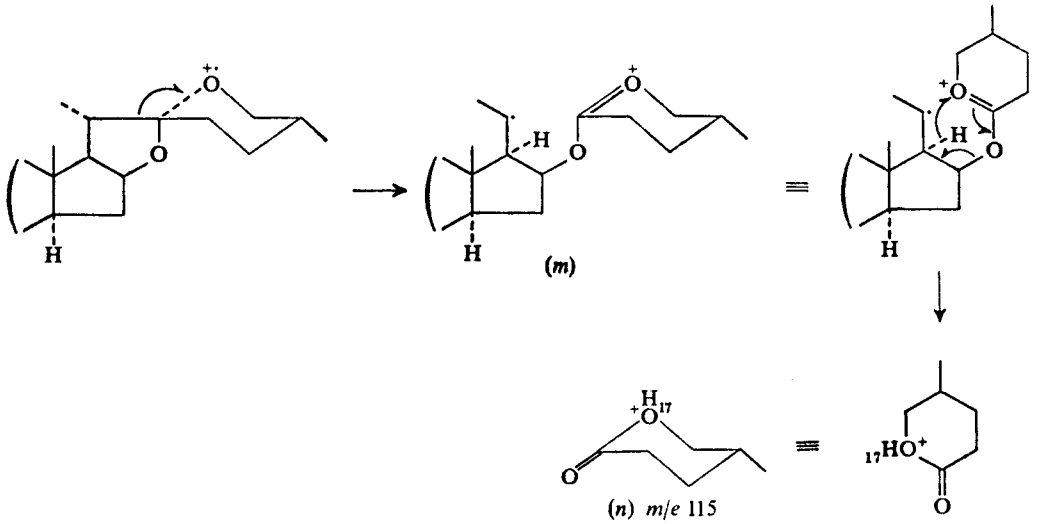


TABLE 2. ISOTOPIC COMPOSITION OF THE MAIN FRAGMENT PEAKS IN THE MASS SPECTRUM OF 15,15,17-d_3-(25R)-5α-SPIROSTAN[a]

m/e	d_0	d_1	d_2	d_3	d_4	d_5
400 [M+][b]	10	30	31	21	6	2
286[b]	11	28	31	21	6	3
271[b]	12	31	33	20	4	—
257[b]	17	37	31	15	—	—
139[b]	49	39	6	6	—	—
126[c]	89	11	—	—	—	—
115[c]	77	23	—	—	—	—

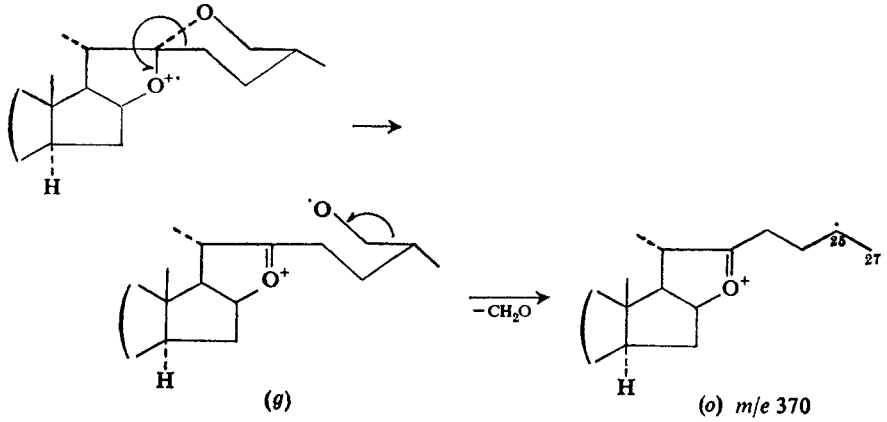
[a] Table 1, footnote *a*.
[b] Calculated from the 70 eV spectrum.
[c] Calculated from the 12 eV spectrum.

That there is little, if any, deuterium at positions 20 through 27 is inferred from the fact that the molecular ion and fragment peaks *m/e* 286, 271 and 257 have essentially the same label; that is, most of the deuterium is located on those positions (C-1 through C-19) still present in the ion of mass *m/e* 257. The synthetic method[11] was expected to provide incorporation only at C-15 and C-17 but *m/e* 126 shows (Table 2) 11% d_1, a figure which can account for the d_4 and d_5 contributions in *m/e* 400 and 286, the presence of d_4 in *m/e* 271, and the existence of d_2 and d_3 in *m/e* 139 by suggesting

* There is possibly a small contribution from a pathway involving the same molecular ion *m* but incorporating a C-15 hydrogen transfer instead of C-17 (see the 15,15-d_2 label, Table 1).

that some deuterium has remained at C-20. Note (Table 2) that *m/e* 257 contains only the expected d_0-d_3 label. Thus, if, from *m/e* 139, we conclude that there is 39% deuterium at C-17 (by assuming the d_2 and d_3 contributions of *m/e* 139 to be a combination of measurement/calculation error* and retained deuterium at C-20), the 23% d_1 in *m/e* 115 is acquired mostly by transfer from C-17 and, thus, the aforementioned fragmentation pattern is operative at least 23/29 × 100% or 59% of the time.

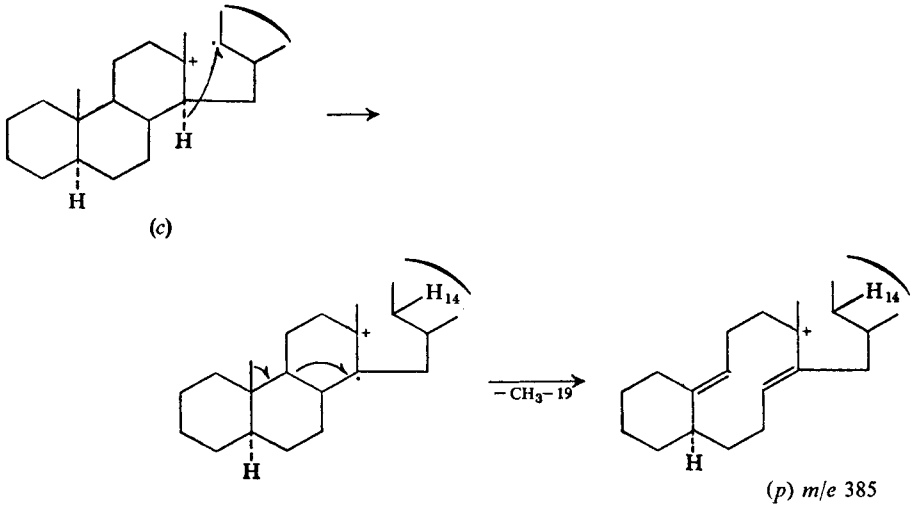
There remain two small peaks at the high molecular weight end of the spectrum (Fig. 1) which deserve passing mention. Loss of 30 mass units gives ion *o* which has been shown to have the composition $C_{26}H_{42}O$ by high resolution. Loss of the C-26 label (see Table 1) means that the neutral species, CH_2O, comes from the ring F oxygen and C-26, a possible structure being *o*.



(*g*) (*o*) *m/e* 370

Ion *p* (*m/e* 385) simply involves loss of a methyl group and, since the labels at C-21 and C-27 are not lost to any noticeable extent, we can only assume that, as in cholestane,[2b] C-19 is preferentially lost, perhaps through the series *c* → *p*.

With the availability of plausible structural and mechanistic proposals for the



(*c*) (*p*) *m/e* 385

* See Table 1, footnote *a*.

principal characteristic fragment ions associated with the steroidal sapogenin ring system typified by (25R)-5α-spirostan (I, Fig. 1), one can now examine the applicability of these results to some concrete problems in sapogenin chemistry.

In connection with the synthesis[11] of the C-23 and C-20 labels, we mentioned the problem of possible incorporation of deuterium at C-25 as would be predicted by a C-25 isomerization mechanism which was offered by Woodward *et al.*,[13] to explain Marker's 'Iso Reaction'[14] and which conformed to the exchange data of Callow and Massy-Beresford.[15] We felt that an attempted confirmation of Callow's[15] data* by

TABLE 3. ISOTOPIC COMPOSITION OF THE MAIN FRAGMENT PEAKS IN THE MASS SPECTRUM OF 20,25-d_2-(25R)-5α-SPIROSTAN[a]

m/e of unlabeled I	d_0	d_1	d_2
400 [M+]	23	68	9
370[b] (*o*)	31	54	15
341 (*i*)	23	76	1
328 (*h*)	21	79	
286 (*d*)	22	78	
271	20	80	
257 (*f*)	97	3	
139 (*b*)	30	63	7
115[c,d] (*n*)	79	19	

[a] Table 1, footnote *a*.

[b] Values very approximate due to low intensity of peak group.

[c] Calculated from 12 eV spectrum. All other values are from the 70 eV spectrum.

[d] Also shows 2% *m/e* 114.

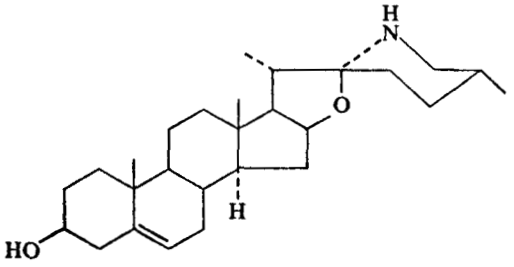
mass spectrometry was in order; thus, (25R)-5α-spirostan (I) in DCl/dioxane (Callow's concentrations) was heated at 100° in a bomb tube for 62½ hrs, after which time the resulting exchanged material was refluxed for one hour in acetic acid to remove the label at C-23. An incorporation of approximately 20% (d_1) at C-25 was determined in the following manner. Table 3 gives the isotopic composition of the main peaks in the spectrum of the exchanged product. Noting again the proposed fragments given in Fig. 1, one can safely assume that no deuterium was incorporated in carbons 1-19 since *f* (*m/e* 257) shows virtually (actually 3%) no deuterium content. Comparison of *d* (*m/e* 286) and 271 with *f* (257) indicates about an 80% incorporation at C-20 and no deuterium at C-21 (286 and 271 have the same isotopic composition). There is no deuterium at C-23 since *h* (*m/e* 328) has the same composition as 286 and 271 and, in a like manner, incorporation at C-24 is rejected by comparison of *i* (*m/e* 341) *vs.* 286. If any deuterium were at C-26, *o* (*m/e* 370) would show less deuterium than the molecular ion; the small intensity of that peak group precludes accurate (±3%) calculation, but an isotopic composition higher than the molecular ion seems to indicate no incorporation at C-26. Thus, since the ion of mass 115 encompasses carbons 22-27 and since we exclude incorporation at C-27,† the

* Callow's conclusions are based on degradation and microanalysis.

† We have no basis for this assumption except that incorporation at C-27 would have to proceed by a mechanism which would put deuterium first at C-26 and, since none is located at C-26 (see above for the isotopic composition of *o*, *m/e* 370), the former is assumed to be all d_0.

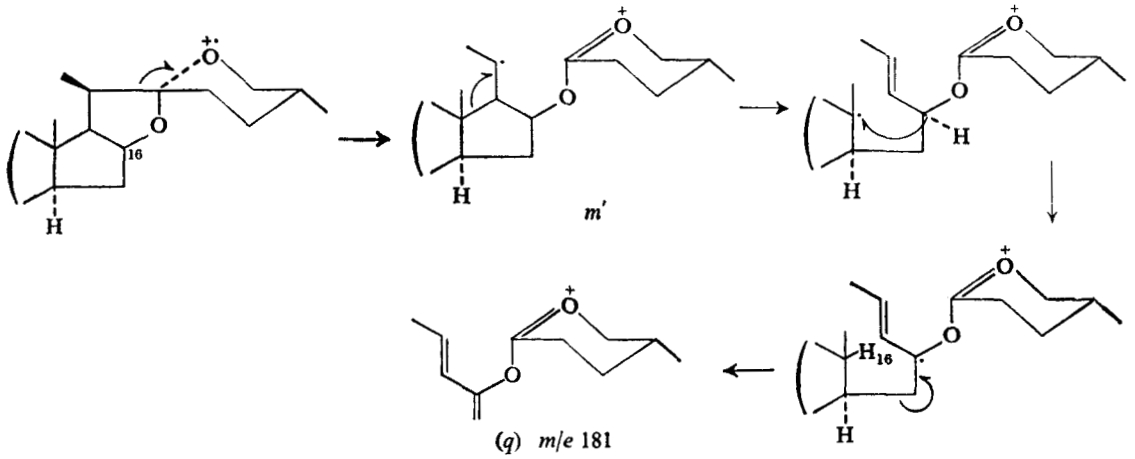
approximately 20% incorporation must be at C-25. The discrepancy from Callow's[15] value of ~50% incorporation at C-25 could perhaps be explained by the fact that he used titogenin (I with a 3β-hydroxyl group) while we used the 3-deoxy compound I and that while both incorporation values rest on results obtained from crystalline material, the crystallization process might have removed more of the 25S isomer (if it were present) in our case than in his.

In another method of application, it has already been noted* that replacement of the ring F oxygen by nitrogen should enhance the abundance of any ion retaining the positive charge at that atom since the charge would be more readily stabilized by the nitrogen atom. Confirmation is given by the spectrum of solasodine (XI)* in which the ions of mass 114, 125 and 138 (corresponding to *n*, *l* and *b* in (25R)-5α-spirostan, Fig. 1) account for most of the total ionization of the compound. The significant peak at *m/e* 113 may easily be rationalized by simple homolytic fission of the C-16, oxygen bond in the ring F nitrogen-containing analog of molecular ion *m*.



(XI)

In the paper[11] describing the synthesis of the labeled compounds, there are also included two structural isomers of (25R)-5α-spirostan (I), (25R)-5α, 14β-spirostan (XII) and (20R, 25R)-5α-spirostan (XIII), the mass spectra of which are shown in Figs. 3 and 4, respectively. Whereas the former shows only quantitative differences from that (Fig. 1) of (25R)-5α-spirostan (I), the latter is not only quantitatively but also qualitatively different, displaying an entirely new doublet at *m/e* 181, 182, shown by high resolution measurements to be $C_{11}H_{17}O_2$ and $C_{11}H_{18}O_2$ respectively. Deuterium



(*q*) *m/e* 181

* Reference 3m, pp. 19, 114 and H. Budzikiewicz, *Tetrahedron* **20**, 2267 (1964).

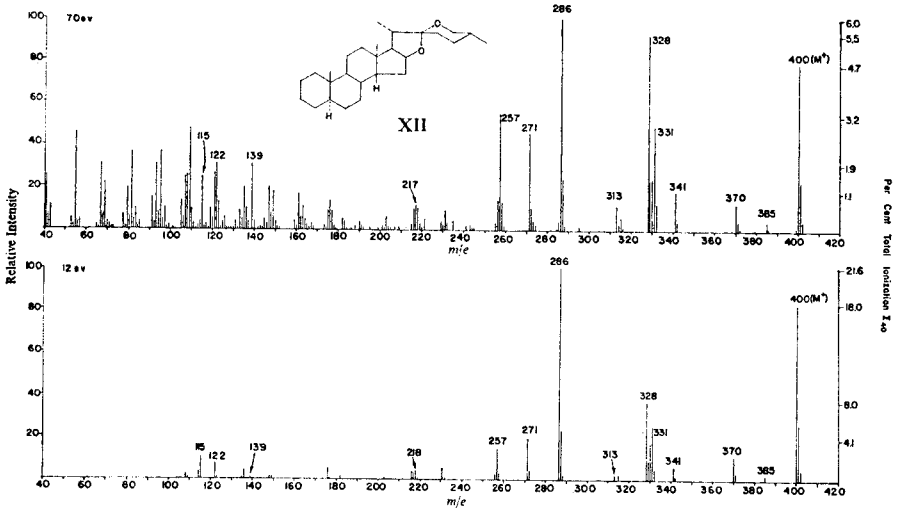


FIG. 3. Mass spectra (70 and 12 eV) of (25R)-5α,14β-spirostan (XII).

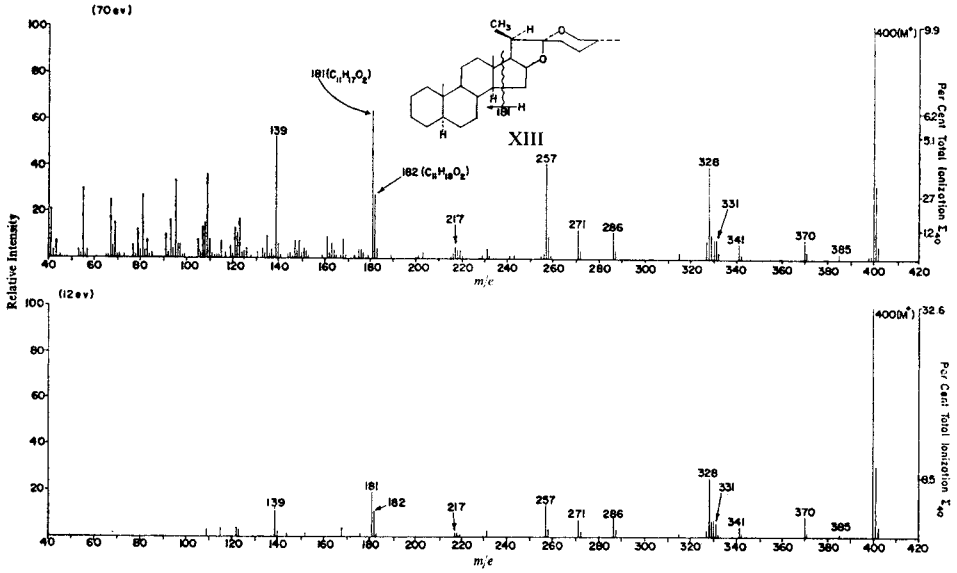


FIG. 4. Mass spectra (70 and 12 eV) of (20R,25R)-5α-spirostan (XIII).

labeling at C-16 in the 20R-isomer XIII confirms that the major fragmentation pathway may be written as $m' \rightarrow q$.

Wall* has suggested that because of steric strain caused by the proximity of C-18 and C-21 in XIII, the compound, upon electron-impact, might first be opening to the furosten analog XIV, the mass spectrum of which is shown in Fig. 5. Indeed, the two are very similar except for the emergence of weak peaks at *m/e* 287 and 302 in the

* Private communication from Dr Monroe E. Wall, Research Triangle Institute, Durham, North Carolina.

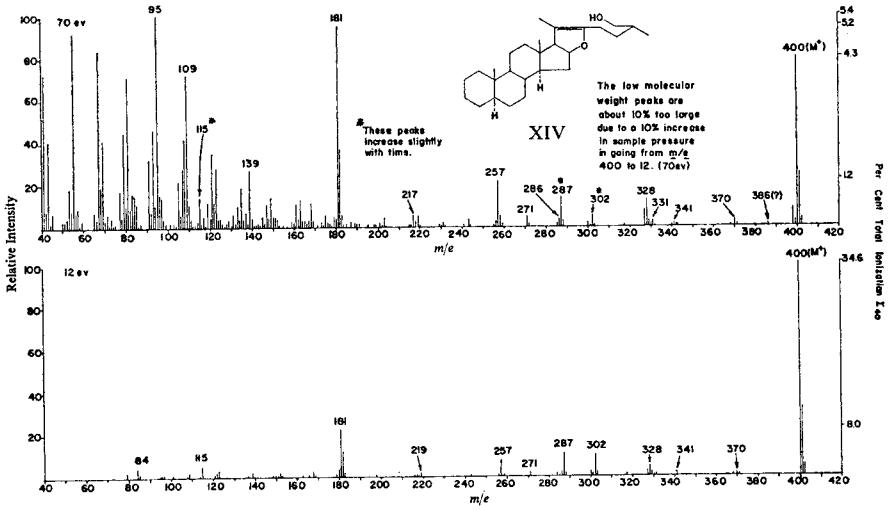
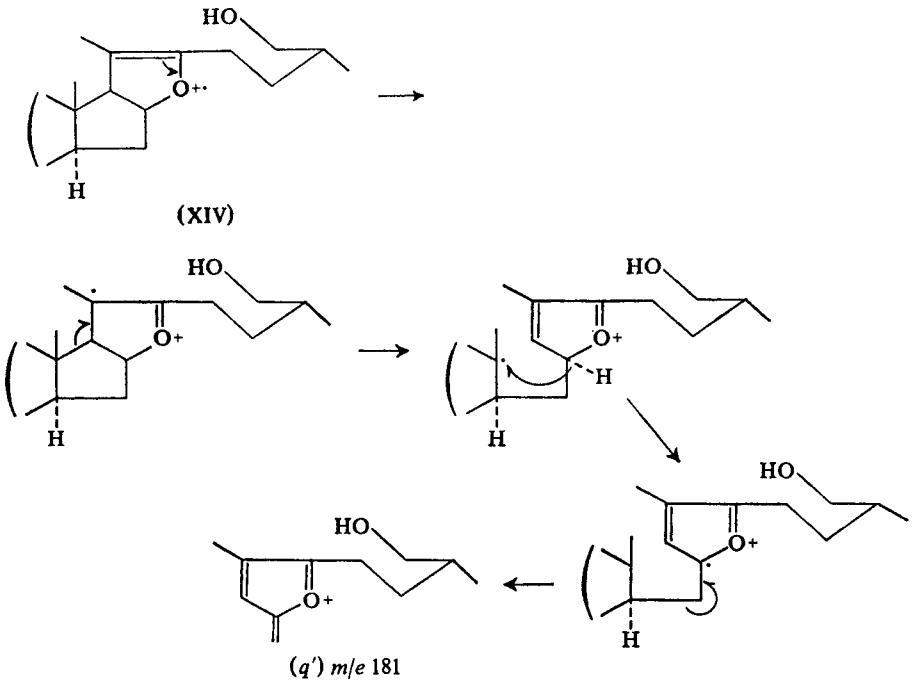


FIG. 5. Mass spectra (70 and 12 eV) of (25R)-5α-furost-20(22)-en-26-ol (XIV).



spectrum (Fig. 5) of XIV. In the case of the furosten XIV, *m/e* 181 may be rationalized in the above manner.

It is also noteworthy that in both XIII and XIV the molecular ion has become quite intense, actually being the base peak at both high and low voltage in the former.* Exactly the same situation holds in the comparison of the spectra (Figs. 6 and 7) of

* Compare with I (Fig. 1).

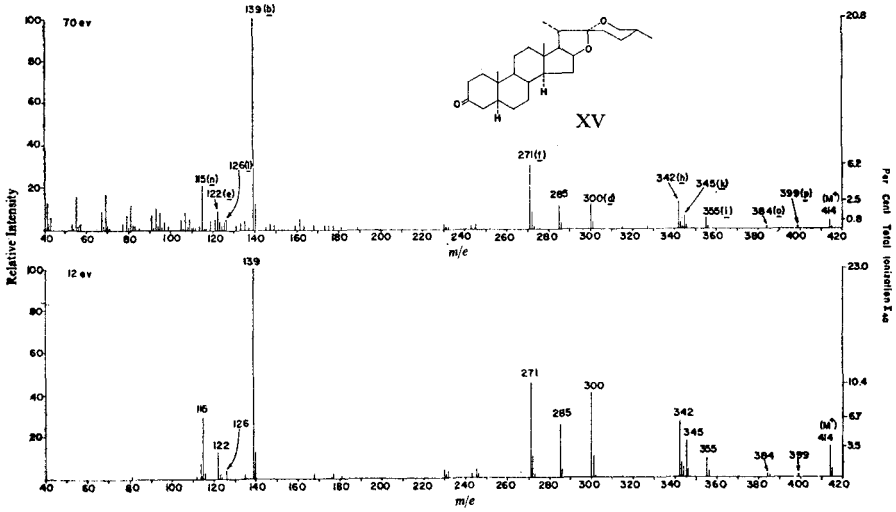


FIG. 6. Mass spectra (70 and 12 eV) of (25R)-5α-spirostan-3-one (XV).

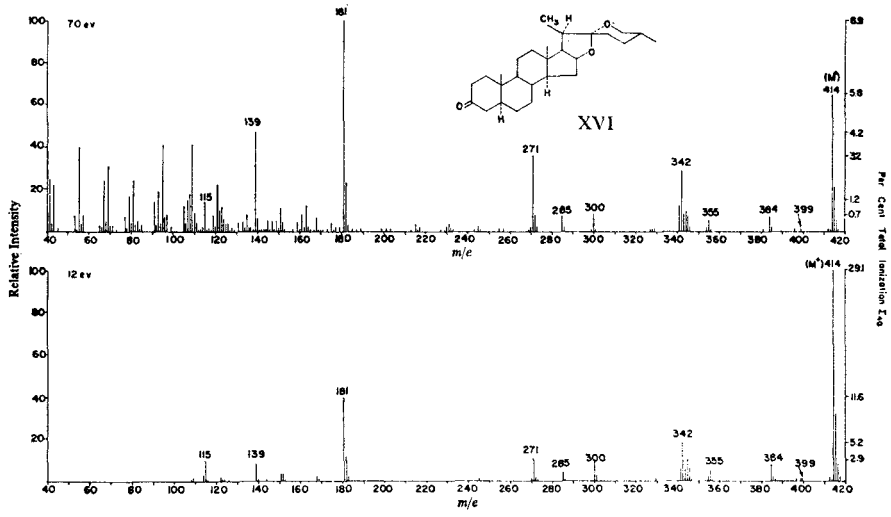


FIG. 7. Mass spectra (70 and 12 eV) of (20R,25R)-5α-spirostan-3-one (XVI).

the C-20 isomeric 3-ketones XV and XVI, the carbonyl group having no effect other than to shift all ring A-containing peaks by 14 mass units.

The presence of olefinic unsaturation is known* to change drastically the course of the main fragmentations in normal steroids. The same is partially true in the steroidal sapogenin as is shown in Figs. 8 to 11 which present the spectra of the four isomeric olefins, the Δ^{14}-(XVII), Δ^{20}-(XVIII), and Δ^{23}-(XIX) (25R)-5α-spirostens and Δ^{24}-5α-spirosten (XX), respectively. No deuterium labeling has been performed in these unsaturated molecules; speculative assignments for the main fragments may be found elsewhere.[16]

* For some examples, see ref. 3a and C. Djerassi, *Pure Appl. Chem.* in press.

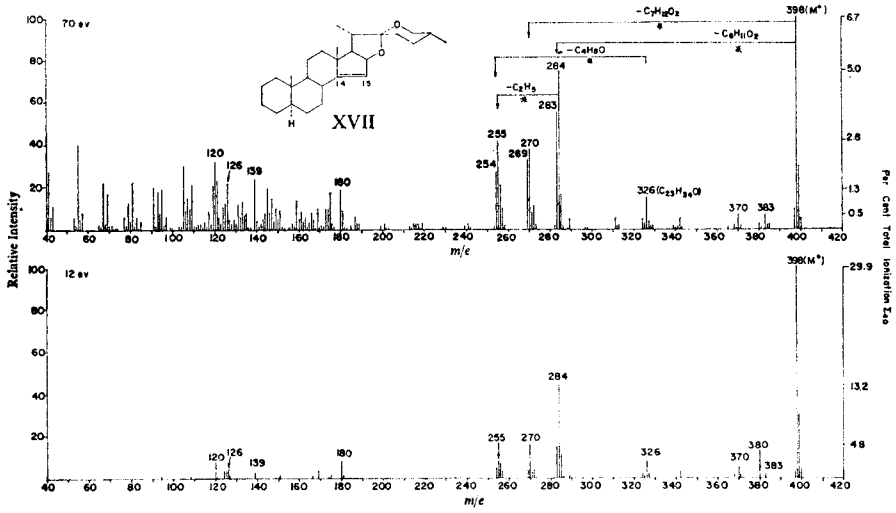


FIG. 8. Mass spectra (70 and 12 eV) of (25R)-5α-spirost-14-en (XVII).

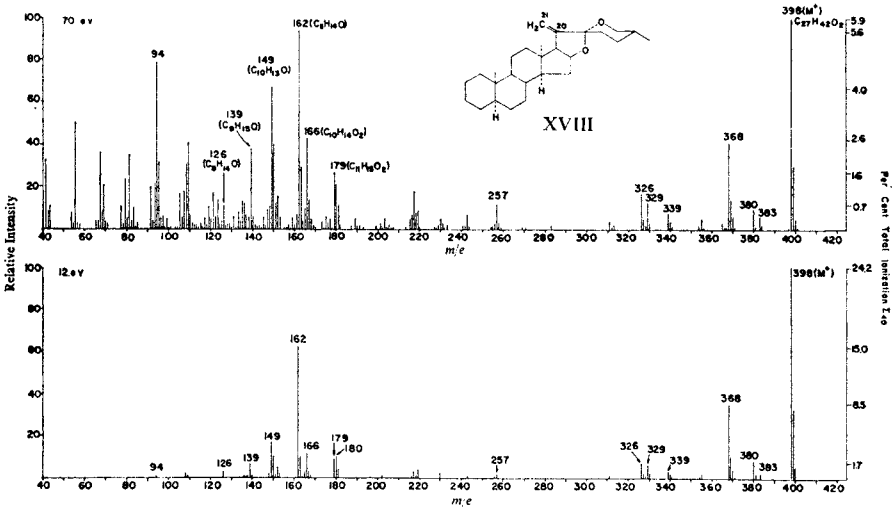


FIG. 9. Mass spectra (70 and 12 eV) of (25R)-5α-spirost-20-en (XVIII).

In theory, a given substituent can be localized fairly precisely in certain portions of the molecule by determining the occurrence of appropriate mass shifts in certain peaks and not in others. That this treatment works well in some cases but not in others may be illustrated by considering the isomeric sapogenin ketones XV and XXI to XXIV, the spectra of which are shown in Figs. 6 and 12–14.*

In the case of the 3-ketone XV (Fig. 7), the peak shift approach works perfectly. The 14 mass unit shift of the molecular ion indicates the presence of a carbonyl

* The spectrum of the 15-ketone XXII is found in ref. 5.

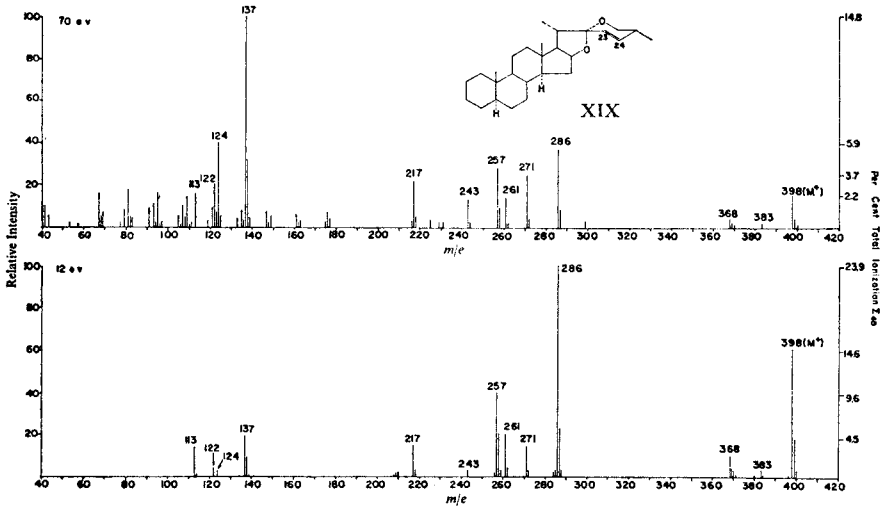


FIG. 10. Mass spectra (70 and 12 eV) of (25R)-5α-spirost-23-en (XXIX).

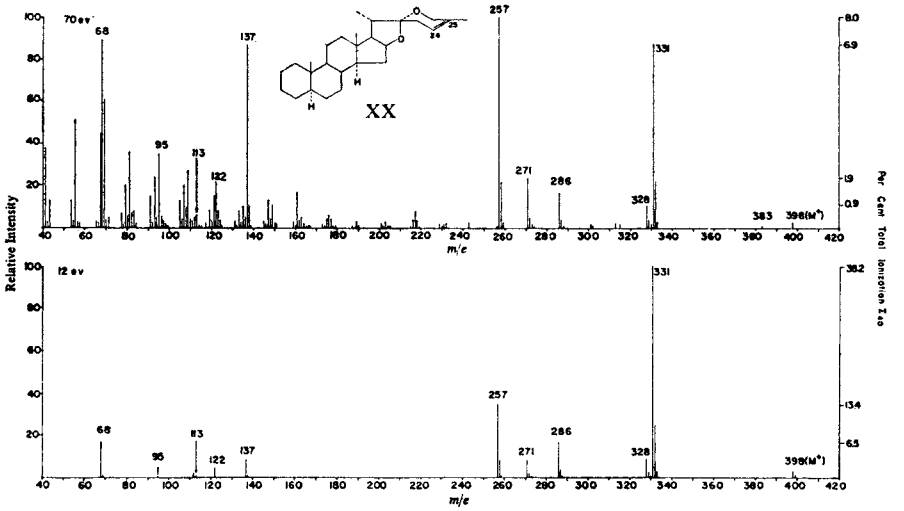


FIG. 11. Mass spectra (70 and 12 eV) of 5α-spirost-24-en (XX).

group while the peaks *m/e* 271, 285, 300, 342, 345 and 355 [appropriately shifted analogs of *m/e* 257 (*f*), 271, 286 (*d*), 328 (*h*), 331 (*k*) and 341 (*i*) in Fig. 1], as compared with ring D-containing fragment *e* (*m/e* 122) and ring F-containing fragments *b* (*m/e* 139) and *n* (*m/e* 115), locate the carbonyl in the ring A-C portion of the molecule.*

In the case of the 12-ketone XXI (Fig. 12), however, this approach fails partly. The spectrum does indeed show the characteristic peak at *m/e* 139 (*b*) as well as peaks

* To locate the carbonyl precisely, the spectra of comparison compounds would have to be available.

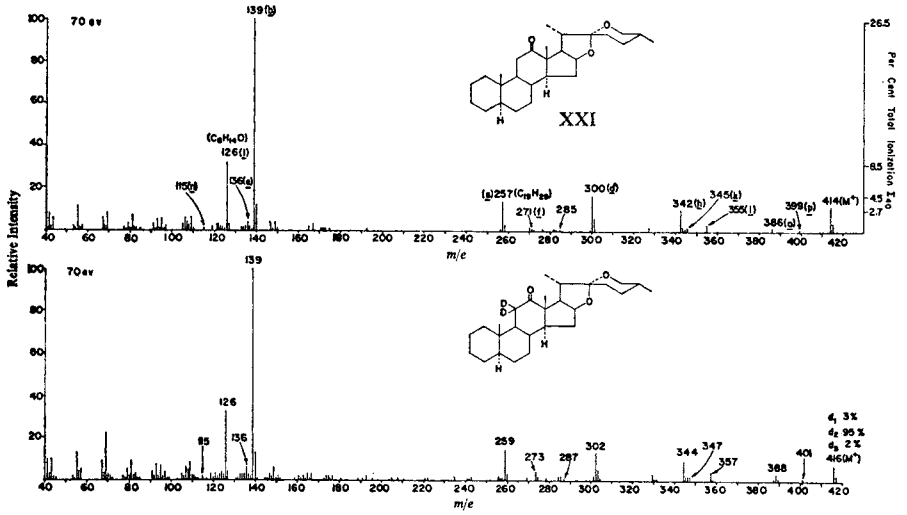


FIG. 12. Mass spectra (70 eV) of (25R)-5α-spirostan-12-one (XXI) and 11,11-d_2-(25R)-5α-spirostan-12-one.

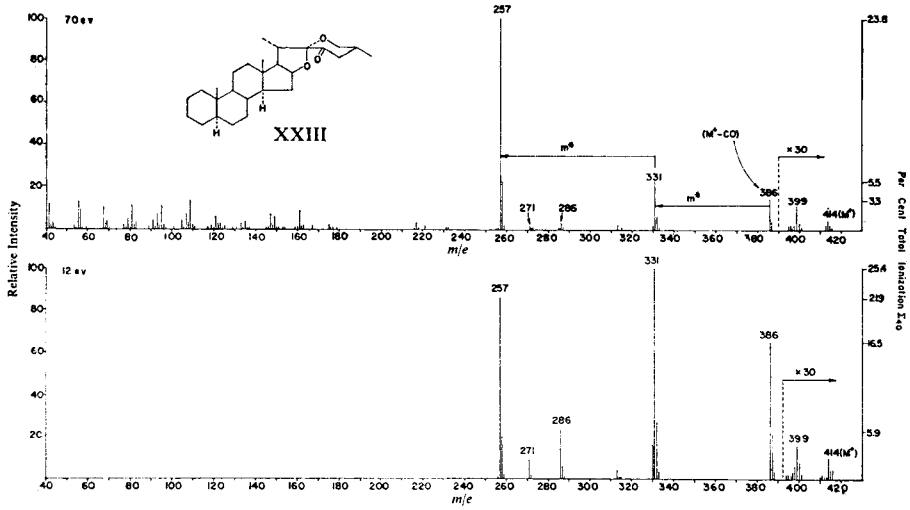


FIG. 13. Mass spectra (70 and 12 eV) of (25R)-5α-spirostan-23-one (XXIII).

at *m/e* 300 and 342 which correspond to *m/e* 286 (*d*) and 328 (*h*) in the (25R)-5α-spirostan (I) spectrum (Fig. 1). There are, however, certain significant differences to which attention should be called.

One difference is the absence in Fig. 12 of all but a very minor *m/e* 115 peak and the absence of strong, appropriately shifted analogs (*m/e* 136, 271, 285 and 345) of the *m/e* 122 (*e*), 257 (*f*), 271 and 331 (*k*) peaks. In at least two cases—*m/e* 122 (*e*) and 257 (*f*)—such absences may be rationalized by the undesirability of placing a carbonyl group next to a positive charge (at C-13), but such an objection should also apply to the *m/e* 286 (*d*) species, yet its counterpart (*m/e* 300) is clearly visible in Fig. 12. This

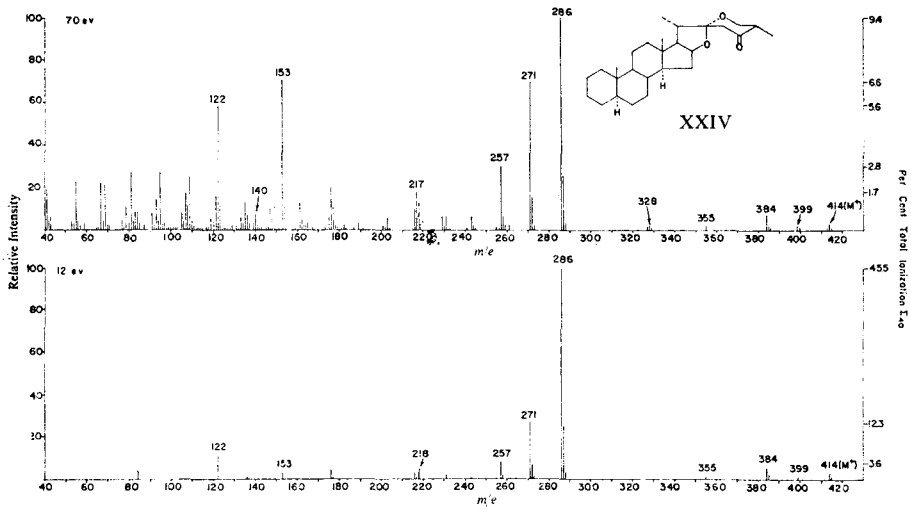
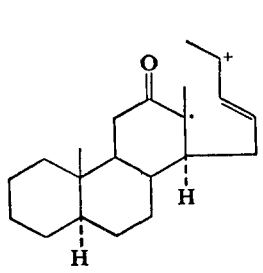
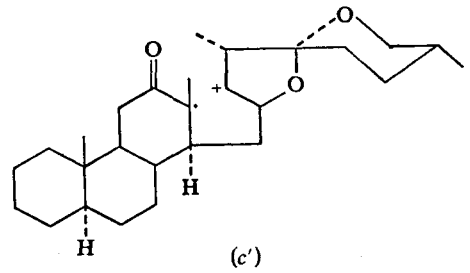


FIG. 14. Mass spectra (70 and 12 eV) of (25R)-5α-spirostan-24-one (XXIV).

apparent discrepancy can, perhaps, be accommodated by attributing the much more favorable canonical form *d'* to the ion of mass 300 in XXI, a similar alternative representation not being possible in the 12-keto analog of *f*, *m/e* 257, because the initial trigger provided by the C-14 hydrogen migration to a radical site at C-17 in molecular ion *c* would not be feasible in *c'*.



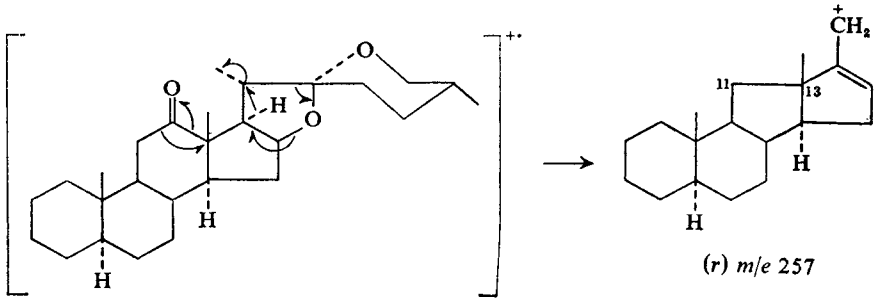
(*d'*) *m/e* 300



(*c'*)

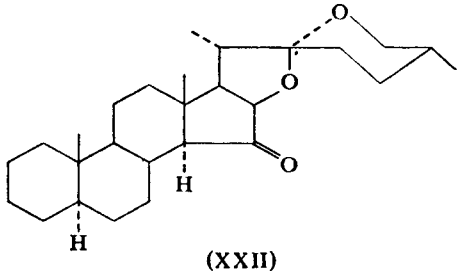
Even more interesting and potentially misleading is the existence of a peak at *m/e* 257* in XXI, since it might be confused with the *m/e* 257 peak in I. Obviously, these two species of identical mass, as confirmed by high resolution, cannot possess the same structure and, since C-11 is retained, as demonstrated by the spectrum of 11,11-dideuterio-(25R)-5α-spirostan-12-one (Fig. 12), the elements of carbon monoxide must have been ejected from ring C. The simplest representation is *r* which requires the postulate of an internal 1,2-hydrogen shift (from C-17 to C-20) for which there seems to exist some precedent in the genesis of *b*, *m/e* 139.

* As expected, this peak appears at *m/e* 273 in the spectrum of hecogenin (XXI with a 3β-hydroxyl group).



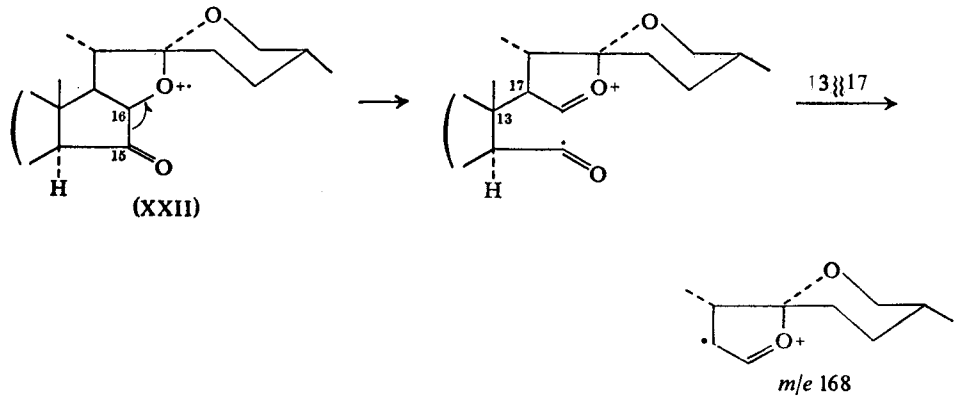
(r) m/e 257

In the 15-keto derivative XXII,* it would be even more difficult to determine the position of the carbonyl without prior knowledge since, of the three strong peaks in



(XXII)

the spectrum, only two, *l* (*m/e* 126) and *b* (*m/e* 139), are equivalent to those in I (Fig. 1). The new peak, *m/e* 168, can be rationalized as follows. Note that the trigger, the fission of the 15,16 bond, gives the acylium radical; without the carbonyl, this would be a primary radical, not a favorable species. The result (*m/e* 168) is not seen in Fig. 1.



(XXII)

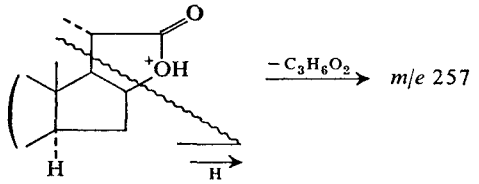
m/e 168

The peak shift approach is also not applicable to the 23-ketone XXIII (Fig. 13) since, in the manner of the 15-ketone XXII, the spectrum depends as a trigger upon the initial formation of an acylium radical analogous to *j* having a doubly bound oxygen at C-23, which leads to loss of CO (*m/e* 386) and then to *k* or *k'* (*m/e* 331) which decomposes to *m/e* 257, not necessarily having the same structure as *f* (Fig. 1).†

* See ref. 5. The MS-9 spectrum is essentially identical.

† There are metastable peaks for *m/e* 386 → 331 and for *m/e* 331 → 257.

Without recourse to deuterium labeling, it seems wise only to indicate formation of *m/e* 257 as



(*k*) *m/e* 331

The 24-ketone XXIV (Fig. 14) conforms quite nicely to peak shift interpretation. While the peaks associated with the opening of ring F are quite weak, the fact that *h* (*m/e* 328) has not shifted and that *i* (*m/e* 341) has shifted to *m/e* 355 would indicate that a carbonyl, indicated from the 14 mass unit shift of the molecular ion to *m/e* 414, is at C-24.* Further evidence for location of the carbonyl in ring F is the shift of *b* (*m/e* 139, Fig. 1) to *m/e* 153 and the retention of *e* (*m/e* 122), *f* (*m/e* 257), 271 and *d* (*m/e* 286) at the same positions.

In addition to comparing the effect on the mass spectrum of I (Fig. 1) of incorporation of olefinic unsaturation, of changes in stereochemistry and of the carbonyl group in different positions of the molecule as described above, we have examined the spectra of many other derivatives of I, notably the isomeric 23 monobromides,[10] the 23-dibromide, the six isomeric 23, 24 and 25 alcohols, and the 23, 24 and 24, 25 epoxides. Due to limitations of space, these will not be reproduced here but can be found elsewhere.[16] Copies of all spectra will be sent to the Mass Spectrometry Data Centre, United Kingdon Atomic Energy Authority, Atomic Weapons Research Establishment, Bldg. A8.1A, Aldermaston, Berkshire, England.

EXPERIMENTAL†

Spectra of all samples were determined by Messrs R. G. Ross (mostly), C. E. Carroll and Dr A. M. Duffield using an AEI MS-9 mass spectrometer with a direct inlet probe and source temperature of 200°, ion current 100 μA. High resolution measurements are accurate to 3 ppm. Deuterium isotope compositions were calculated from peak heights measuring directly from the original spectra, corrected for naturally occurring ^2H, ^{13}C and ^{18}O, and are reliable to 3% or better as determined by successive trials on measurements of (25R)-5α-spriostan (I). Final values were determined by mathematical comparison of labeled and unlabeled spectra; the technique is described in detail elsewhere.[16]

Melting points are uncorrected and were determined on the Kofler Block. The infrared spectra were run in chloroform on a Perkin–Elmer 421 Grating Spectrophotometer using sodium chloride cavity cells. Optical rotations were also done in chloroform. The n.m.r. spectra were run in chloroform (pretreated with Na_2CO_3) on a Varian HA-100 instrument by Drs Lois Durham and M. Bramwell. R.t.l.c. = Repetition thin layer chromatography, the term we have given to preparative t.l.c. when the plate is run more than once with a thorough drying in between. Plates contained a layer of 1 mm thick silica gel HF-254 (E. Merck AG, Darmstadt)—sample application by the method of H. J. Monteiro, [*J. Chromatog.* **18,** 594 (1965)]—detection by 'hot wire' [simplified version of that of J. L. Bloomer and W. R. Eder, *J. Chromatog.* **34,** 548 (1968)]. The solvent mixtures are denoted in parts per hundred and number (N) of times run by Nx. All microanalyses are by Messrs E. Meier and J. Consul.

* The peak *k* (*m/e* 331) does appear in the original spectrum but is so small as to be less than one unit high on a plot with *m/e* 286 = 100. Peaks <1% of base peak are not usually plotted.

† The synthesis of all compounds mentioned in this work except for X, XVI and the DCl/dioxane exchange, which are included here, may be found in ref. 11.

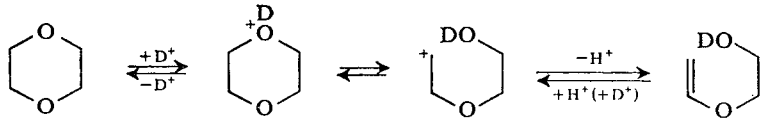
20,25-d_2-(25R)-5α-*Spirostan* (see Table 3):—(25R)-5α-Spirostan (I, 100 mg, 0·25 mmole) and 6·25 ml 0·5*N* DCl/dioxane* (3·125 mmole acid, 12·5 molar excess) were sealed in a bomb tube and heated in a steam bath for 62·5 hrs. After neutralizing the reaction by partitioning between ether/5% sodium carbonate, the resulting organic layer was washed well with water, dried ($MgSO_4$), evaporated *in vacuo* and crystallized from ether/methanol to give 66 mg of white crystals, m.p. 166·5 to 169°; d_1 9%, d_2 42%, d_3 43%, d_4 6% (*ca.* 80% incorporation at C-20, calculated in same manner as results of Table 3). This material (50 mg) was refluxed in 15 ml glacial acetic acid for one hr to remove deuterium from C-23. Evaporation of the solvent *in vacuo* left a white residue which was partitioned between ether and 5% sodium bicarbonate and the organic layer washed with bicarbonate and water, dried ($MgSO_4$) and evaporated *in vacuo* giving a residue which, after crystallization from ether/methanol, afforded 38 mg of 20,25-d_2-(25R)-5α-*spirostan*, m.p. 167 to 171° (see Table 3).

(25R)-5α-*Spirostan* 3-*ethylene ketal* (X): A solution of (25R)-5α-spirostan-3-one (XV, 130 mg) in dry benzene (10 ml) containing ethylene glycol (0·5 ml) and *p*-toluenesulfonic acid monohydrate (10·4 mg) was refluxed for 21½ hrs, using calcium chloride (changed frequently) in a Soxhlet extractor to remove water. The solution was partitioned between ether and 10% sodium bicarbonate and the ether phase washed with 10% sodium bicarbonate and water, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by R.t.l.c. [2*x*, EtOAc (7)/benzene (93)] and crystallized from ether/methanol giving (25R)-5α-*spirostan* 3-*ethylene ketal* (X, 97 mg, 62%): flakes, m.p. 271·5 to 272·5°; ν_{max} 1170, 1150, 1129, 1095, 1061, 1040, 1014, 1002, 973, 941, 914, 891, 858 cm^{-1}; n.m.r. δ 0·760 (*s*, CH_3-18), *ca.* 0·79 (*d*, CH_3-27, lies under the C-18 and C-19 resonances), 0·822 (*s*, CH_3-19), 0·952 (*d*, J = 6·6 Hz, CH_3-21), 3·90 (*s*, $2CH_2$ ketal); $[\alpha]_D^{24°}$ −50° (*c* 0·685). (Found: C, 75·78; H, 10·10; Mol. wt. 458 (mass spec). $C_{29}H_{46}O_4$ requires C, 75·94; H, 10·11%; Mol. wt. 458·66).

C-20 *Isomerization of* (25R)-5α-*spirostan*-3-*one* (XV):† In a dry atmosphere, methylamine hydrochloride (220 mg) was added to a refluxing solution of the ketone XV (645 mg) dissolved in pyridine (4 ml)/acetic anhydride (4 ml, distilled from sodium). Reflux was continued for 2 hrs. and the solution then partitioned between ether and water. The organic layer was washed with 10% sodium bicarbonate, 2% HCl, again with bicarbonate, and water, dried ($MgSO_4$), and evaporated *in vacuo*. The residue, a yellow semi-solid, was dissolved in basic methanol (50 ml, 1·4 gm potassium hydroxide) and refluxed for 1 hr, giving a bright red solution which, with cooling and stirring, was neutralized with glacial acetic acid (1·5 ml) giving a precipitate and a return to the original yellow color. The precipitate dissolved upon addition of more acetic acid (50 ml) and the acidic solution was allowed to stand at room temperature for 60 hrs, after which it was partitioned between ether and water. The aqueous layer was extracted with ether and the combined organic layers washed with 10% sodium bicarbonate and water, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by R.t.l.c. [4*x*, EtOAc (10)/*n*-hexane (90), 2 drops pyridine/100 ml solution] giving four compounds (R_f 0·50, 0·45, 0·37, 0·30) all of which were crystallized from acetone. Analytical samples were also from acetone; yields below denote crystalline material after the first crystallization.

(25R)-5α-*Spirostan* 3,3-*dimethyl ketal* (R_f 0·50, 64 mg): needles, m.p. 184 to 186·5°; ν_{max} 1168, 1147, 1127, 1093, 1064, 1040, 972, 913, 888, 857 cm^{-1}; n.m.r. δ 0·761 (*s*, CH_3-18), 0·807 (*s*, CH_3-19), CH_3-27 is hidden under the C-18 and C-19 resonances, 0·955 (*d*, J = 6·5 Hz, CH_3-21), 3·13 and 3·18 (*s*, 2-OCH_3); $[\alpha]_D^{26·5°}$ −54° (*c* 1·100, dioxane). (Found: C, 75·85; H, 10·58; Mol. wt. 460 (mass spec.). $C_{29}H_{48}O_4$ requires C, 75·60; H, 10·50%; Mol wt. 460·67).

* The reagent was prepared by bubbling DCl gas (Stohler Isotope Chemicals) into dioxane (distilled from sodium/benzophenone and again, under vacuum, from lithium aluminum hydride). Standardization by classical titration. Note that dioxane is really a poor solvent for this reaction since protons can enter the medium through the reaction



We have used this solvent in order best to reproduce the literature.[15]

† We wish to thank Dr Monroe E. Wall, Research Triangle Institute, Durham, North Carolina, for his suggestion of this method.

(20R,25R)-5α-*Spirostan* 3,3-*dimethyl ketal* (R_f 0·45, 30 mg): fine needles, m.p. 185 to 186°* [mixed m.p. with the ketal above, 181 to 186°]; ν_{max} 1159, 1131, 1090, 1060, 1037, 1008, 963, 918, 891, 868, 854 cm^{-1}; n.m.r. δ *ca.* 0·775 (*d*, J = 5·8 Hz, CH_3-27 partially hidden by C-19 resonance), 0·788 (*s*, CH_3-19), 0·925 (*s*, CH_3-18), 1·128 (*d*, J = 8·0 Hz, CH_3-21), 3·13 and 3·19 (*s*, 2-OCH_3); $[\alpha]_D^{26\cdot4°}$ −45° (*c* 0·667, dioxane). (Found: C, 75·54; H, 10·47; Mol. wt. 460 (mass spec.). $C_{29}H_{48}O_4$ requires C, 75·60; H, 10·50%; Mol. wt. 460·67).

The third band (R_f 0·37) was identified as the starting material (XV, 184 mg): rectangular plates, m.p. 198 to 203·5°; (compare ir spectrum with compound R_f 0·30 below) ν_{max} 1706 (C=O), 1172, 1152, 1122, 1093, 1069, 1043, 1018, 975, 952, 915, 891, 860 cm^{-1}; $[\alpha]_D^{25\cdot5°}$ −44° (*c* 1·566, dioxane). [lit.[17] m.p. 203 to 205°; $[\alpha]_D^{35°}$ −53° (*c* 0·83 $CHCl_3$)].†

(20R,25R)-5α-*Spirostan*-3-*one* (XVI, R_f 0·30, 64 mg): m.p. 174 to 180* (sublimed at 10^{-5} mm Hg, 124°); ν_{max} 1706 (C=O), 1182, 1161, 1130, 1067, 1036, 1010, 967, 953, 917, 891, 868, 853 cm^{-1}; n.m.r. δ 0·787 (*d*, J = 5·8 Hz, CH_3-27), 0·966 (*s*, CH_3-18), 1·016 (*s*, CH_3-19), 1·146 (*d*, J = 8·0 Hz, CH_3-21); $[\alpha]_D^{25\cdot2°}$ −63° (*c* 0·172, dioxane). (Found: C, 77·93; H, 10·21; Mol. wt. 414 (mass spec.). $C_{27}H_{42}O_3$ requires C, 78·21; H, 10·21%; Mol. wt. 414·61).

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† For the n.m.r. spectrum of this compound see K. Tori and K. Aono, *Ann. Rep. Shionogi Res. Lab.* **14,** 136 (1964).